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Molecular Detection of Specific Virulence Genes of Escherichia Coli Isolated from Urinary Tract Infections in AL-Najaf, Iraq

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Abstract

The present study was carried out at AL-Najaf, AL-Ashraf Teaching Hospital. A collection of 150 urine samples was acquired from individuals who were suspected of having urinary tract infections, as determined by their clinical manifestations and symptoms as diagnosed by the attending physician. Subsequently, the samples underwent analysis utilizing optical microscopy and bacterial cell detection techniques, yielding definitive evidence regarding the presence of pathogenic bacteria. In order to identify the genes responsible for specific virulence factors, the positive samples underwent culturing and were subsequently analysed using the polymerase chain reaction (PCR) technique. Among the 150 samples analysed, a total of 110 specimens, accounting for 74.33% of the sample population, exhibited noteworthy bacterial growth. Specifically, 81 specimens, representing 73.64% of the female specimens, displayed significant bacterial growth, while the remaining 29 specimens, constituting 26.36% of the male specimens, demonstrated the same characteristic. The DNA was isolated from *E. coli* isolates using the DNA isolation kit. The utilization of the PCR was employed in order to ascertain the existence of particular genes, specifically *16s RNA, LuxS, Stx2a, HlyA, and RopE*. The findings of the study indicated that all of the isolates (100%) were found to possess the *16s RNA* gene, the *RopE* gene and the *LuxS* gene. Additionally, a smaller proportion of the isolates (20%) were found to carry the *hlyA* gene, while a minority of the isolates (5.7%) were found to carry the *Stx2a* gene.

Keywords: *Escherichia Coli*, Urinary Tract Infection, *16S rRNA*, *HlyA*, Virulence Genes

Introduction

Urinary tract infections (UTIs) are a prevalent health issue, impacting a substantial number of individuals annually on a global scale, with an estimated incidence of 150 million cases per year (Tamadonfar *et al.*, 2019) [26]. As stated by Medina and Castillo-Pino (2019) [19], it is estimated that approximately 60% of women will encounter at least one UTI throughout their lifespan. UTI is a broad term employed to characterize infections that arise from the colonisation of pathogens within any component of the urinary system. The aforementioned conditions encompass cystitis, pyelonephritis, renal abscess, urethritis, and prostatitis. Within the domain of clinical practice, UTIs are categorized into two discrete classifications: uncomplicated and complicated. Uncomplicated urinary tract infections (UTIs) consist of two separate entities: acute uncomplicated cystitis (AUC), which pertains to an infection occurring in the bladder or lower urinary tract, and acute uncomplicated pyelonephritis (AUP), which refers to an infection that specifically targets the kidney or upper urinary tract. These conditions are specifically observed in individuals who possess a normal, unobstructed urinary tract and have not undergone any recent instrumentation procedures. Complex urinary tract infections can manifest in individuals who have urinary tracts that display metabolic, functional, or structural abnormalities. UTIs have the potential to affect various components of the urinary tract. One significant outcome of UTIs is a substantial elevation in the likelihood of therapeutic failure (Fazly Bazzaz *et al.*, 2021; Goździkiewicz *et al*) [10, 11]. Escherichia coli *(E. coli*) is a bacterium characterized by its rod-shaped morphology and Gram-negative cell wall structure. It is taxonomically classified as a member of the family Enterobacteriaceae within the class Gamma proteobacteria. According to Jang *et al*. (2017) [15], this particular bacterium exhibits a rapid growth rate when provided with favorable growth conditions, with a replication time of approximately 20 minutes. The initial isolation of this substance was conducted by Theodor Escherichia in 1885, using infant stool as the primary source for characterization (Deb *et al*. 2021) [8]. *E. coli* is one of the common causes of bacterial infections in patients with chronic kidney disease (CKD), which is associated with oxidative stress (Aziz *et al*., 2020). The aim of this study was to investigate the prevalence and distribution of some virulence genes of Escherichia coli isolated from urinary tract infections in AL-Najaf, Iraq.

Materials and Method

Samples

A total of 150 midstream urine samples was procured from patients who had been diagnosed with urinary tract infections at Al-Najaf Al-Ashraf Teaching Hospital, situated in Al-Najaf, Iraq. The time period under consideration spans from September 2022 to November 2022. The diverse samples were subjected to cultivation on three distinct types of agar media, specifically Blood agar, MacConkey agar, and Eosin methylene blue agar. The validation of the differentiation between *E. coli* and other lactose fermenter Enterobacteriaceae is achieved through the implementation of biochemical tests, which involve the evaluation of specific indicators to determine their presence or absence. *E. coli* is identified by obtaining positive results in the methyl red and indole tests, while yielding negative results in the Voges-Proskauer, Simmons citrate, and urease tests. The conclusive diagnosis was established by employing biochemical tests and the Vitek 2 System.

Bacterial DNA Excretion

The process of isolating bacterial chromosomal DNAs was performed using the solar bio DNA isolation kit, following the manufacturer's provided protocol. Following that, the DNAs that were obtained were then subjected to electrophoresis utilizing a gel composed of 1.5% agarose, which had been stained with Gel Red. Subsequently, the gel obtained was subjected to visualization using a UV trans illuminator.

PCR Analysis

PCR technique was employed to analyse the presence of virulence factor genes, namely *16s RNA, Lux S, Hemolysin Alpha, Stx2a, and RopE* genes, the process of amplifying DNA fragments in Escherichia coli is achieved through the utilization of specific primers. The PCR amplification protocol was performed utilizing a thermal cycler (Techne) and employing specific primers that corresponded to the genes enumerated in Table 1.

Table 1: Primers information

Primer Name		Sequence (5'-3')	Product Size (bp)	Reference
16s-rna	F	CTTAACAAACCGCCTGCGTG	103	This study
	R	AAGAAGCACCGGCTAACTCC		
LuxS	F	GTCTTCCATTGCCGCTTTCC	169	This study
	R	TACCCTGGAGCACCTGTTTG		
hlyA	F	ATAGTCACTCCCCGTTCGGT	126	This study
	R	TGTCAGGACGGCAGATGAAC		
Rop E	F	GAAGATACGTGAACGCACG	88	This study
	R	CACCTTACGGGAGCTGGATG		
Stx2a	F	TCTCCCCACTCTGACACCAT	85	This study
	R	AGACGTGGACCTCACTCTGA		

The total volume of each PCR reaction was 20µL. The PCR mixtures for each gene were prepared following the instructions outlined in Table 2. Prior to use, the mixtures were homogenized using a vortex. The PCR programmed was executed in accordance with the thermal programmed specified in Table 3.

Table 2: The components of conventional PCR

S. No	PCR reaction mixture	Volume
1	EntiLink PCR master mix	10 µl
2	Primer forward (10µM)	1 µl
3	Primer reverse (10µM)	1 µl
4	DNA template	2 µl
D.W up to		20 µl

Table 3: The thermal cycling conditions

Stage	Temperature	Time	Number of cycle
Initial denaturation	95	3-5min	1
Denaturation	95	30sec	30 cycles
Annealing	60	30 sec	
Extension	72	30	
Final elongation	72	5 min	1

Results

A total of 150 specimens were collected from Al-Ashraf Teaching Hospital in Al-Najaf province, specifically from male and female patients diagnosed with urinary tract infection. The collection period spanned from September 2022 to November 2022. Among the 150 specimens analysed, a total of 110 specimens (74.33%) exhibited noteworthy bacterial growth. Specifically, 81 specimens (73.64%) were derived from female subjects, while the remaining 29 specimens (26.36%) originated from male subjects, as indicated in Table 4.

Table 4: Distribution and percentages of infection according to gender

Type of patient	Number of Sample	Percentage %	Number of Sample that bacterial growth appeared	Percentage %
Female	103	68.67%	81	73.64%
Male	47	31.33%	29	26.36%
Total	150	100%	110	100%

In this study, we identified multiple bacterial species as the causative agents of urinary tract infections, with *E. coli* being the most prevalent (35 isolates). We extracted genomic DNA from these isolates and tested them for the *16s RNA, Lux S, RopE, hlyA*, and *Stx2a* genes. All isolates (100%) were positive for the *16s RNA* and *Lux S* genes, and the *RopE* gene was present in all isolates as well. The *hlyA* gene was detected in only 20% of the isolates, while the S*tx2a* gene was found in all *E. coli* isolates (5.7% of the total).

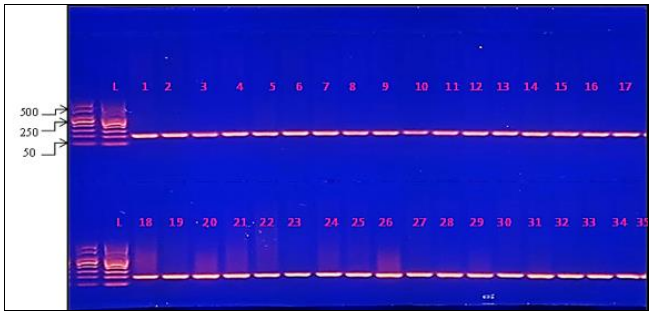

Fig 1: PCR amplification products of *E. coli* isolates that amplified with *16S RNA* gene primers with product 103 bp Lane (L), DNA molecular size marker (50-bp ladder), Lanes (1to35) showed positive results with the *16S RNA* gene

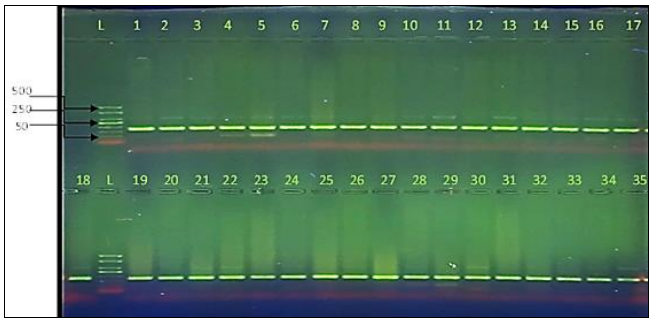

Fig 2: PCR amplification products of *E. coli* isolates that amplified with *LUXS* gene primers with product 116 bp Lane (L), DNA molecular size marker (50-bp ladder), Lanes (1to35) showed positive results with the *LUXS* gene

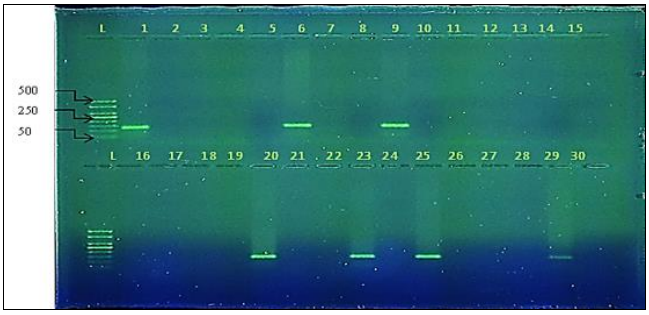

Fig 3: PCR amplification products of *E. coli* isolates that amplified with *HlyA* gene primers with product 126 bp Lane (L), DNA molecular size marker (50-bp ladder), Lanes (6, 9, 20, 23, 25, 29) showed positive results with the *HlyA* gene

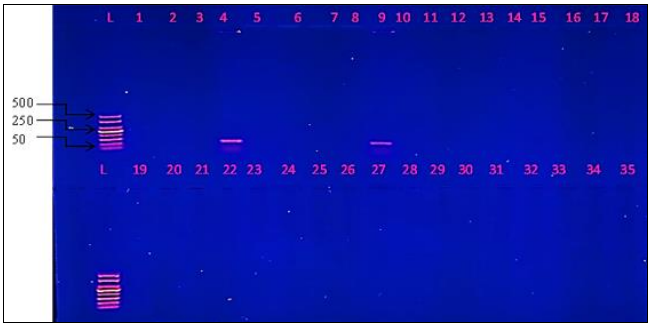

Fig 4: PCR amplification products of *E. coli* isolates that amplified with *STX2a* gene primers with product 85 bp Lane (L), DNA molecular size marker (50-bp ladder), Lanes (4, 9) showed positive results with the *STX2a* gene

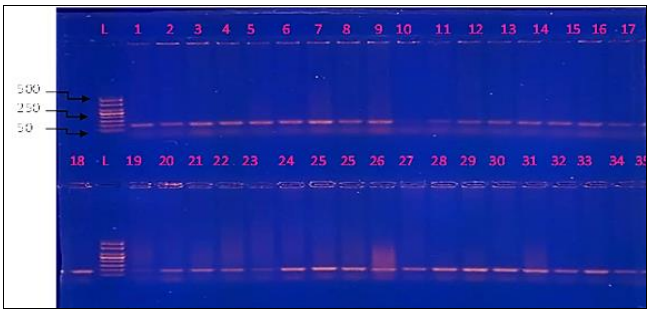

Fig 5: *RopE* gene of *E. coli* isolates that amplified with primers with product 89 bp Lane (L), DNA molecular size marker (50-bpladder), Lanes () showed positive results with the *RopE* gene

Discussion

The findings of present study indicated that the infection ratio among females was higher than males and that can be considered as an acceptable outcome. Women are more vulnerable than men to urinary tract infections due to anatomical factors such as having a shorter urethra, lacking prostatic secretion, being pregnant, and experiencing easier contamination of the urinary tract with faecal bacteria (Awaness *et al*., 2000) [5]. *E.coli* is the most common bacterial pathogen causing UTI in Al-Najaf Al-Ashraf, and it may carry various resistance genes (Hussein *et al*. 2022) [14]. The analysis of gene distributions revealed that the *16S ribosomal RNA* gene, which is used to identify *E. coli* bacteria, was present in all the examined isolates. Following this, the *Lux S* and *RopE* genes were observed to be relatively more prevalent, while the *hlyA* gene demonstrated a lower abundance. Notably, the *Stx2a* gene exhibited a lesser frequency compared to the other four genes. The 16s rRNA molecule is an essential component of the ribosome's structure. According to Reeve (2013) [23], the large subunit of prokaryotes consists of 5S and 23S species, while the small subunit contains a 16S rRNA species. The *16S rRNA* gene exhibits regions of high conservation across all prokaryotic organisms, as well as variable regions that have proven valuable in providing taxonomic insights at various levels of classification and facilitating differentiation between species and isolates (Darwish *et al.*, 2004) [7].

The study conducted by Niu *et al.* (2013) [22] demonstrated that *luxS* has a significant impact on the development of *E. coli* biofilms, regardless of the presence of autoinducer-2, and can aid in the organism's ability to adapt to diverse environmental conditions. The findings of the present study align with those of Hamdi *et al*. (2021) [12], who diagnosed the presence Lux S in the samples isolated from Iraqi pregnant women with UTI. Also, the results of the current study align with the findings of Tiba *et al.* (2008) [27], wherein they observed that 25.3% of their isolates exhibited a positive test result for the *hlyA* gene. The results presented in this study exhibit a strong correlation with the research conducted by Yun *et al.* (2014) [28], wherein it was observed that 20.3% of their isolates were found to possess the *hlyA* gene. The study conducted by Aljebory and Mohammad (2019) [3] revealed that 25.4% of the isolates examined displayed the observed characteristic. In contrast, Allami *et al.* (2022) [4] documented a percentage of 15%, which is comparatively lower than the results obtained in the current study. In contrast, the findings of Johnson and Stell (2000) [16] exhibited slight divergence, they unveiled that 41% of the examined isolates exhibited a positive presence of the hlyA gene. This is in contrast to the results reported by Moeinizadeh and Shaheli (2021) [20], where they observed a higher prevalence of 50% hlyA-positive isolates in their own research. One of the genes we examined was *STX2a*, which encodes a toxin that causes severe intestinal and systemic complications. We found that STX2a was rare among our isolates, which is consistent with most previous studies (Adeli *et al*., 2013; Abbasi and Tajbakhsh, 2015) [2, 1]. However, we found a discrepancy with the study by Mansouri *et al*. (2015) [18], who did not detect STX2a in any of their isolates. This may be due to different methods, sources or regions of the isolates. Shiga toxin-producing Escherichia coli (STEC) are responsible for causing a range of severe illnesses, such as foodborne illnesses, dysentery, hemorrhagic colitis, and hemolytic–uremic syndrome (HUS). The preponderance of cases involving hemorrhagic colitis, hemolytic uremic syndrome (HUS), and sudden death in various age demographics can be attributed to the presence of serotypes 0157:H7. These serotypes are widely

acknowledged as the most consequential strains of Shiga toxin-producing Escherichia coli (STEC) (Mora *et al*., 2007; Santaniello *et al*., 2007) [21, 24].

Rop, also referred to as repressor of primer or RNA one modulator (ROM), is a compact dimeric protein that plays a crucial role in regulating the copy number of ColE1 family and related bacterial plasmids in *Escherichia coli*. Its mechanism involves enhancing the rate of pairing between the preprimary RNA, known as RNA II, and its complementary antisense RNA, RNA I, thus contributing to the maintenance of low plasmid copy numbers (Del Solar and Espinosa, 2000) [9]. We found that *Rop* gene was common among our isolates, which suggests that plasmid maintenance may be important for *E. coli* survival and adaptation. *Rop* gene is a small protein that forms a four-helix bundle structure, which has been used as a model for protein design studies (Schug *et al*., 2007; Kresse *et al*., 2001) [25, 17].

Conclusion

In conclusion, this study revealed the high prevalence and diversity of bacterial species causing urinary tract infections in Al-Najaf province, Iraq. *E.coli* was the most common pathogen, and it exhibited various virulence genes that may contribute to its pathogenicity and persistence in the urinary tract. The detection of these genes may help in the diagnosis and treatment of UTI caused by E.coli. Further studies are needed to explore the molecular mechanisms and epidemiology of these virulence factors in *E. coli* and other bacterial species.

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